Application of Response Surface Methodology for Removal of Remazol Yellow (RR) by Immobilised *S. cerevisiae* on Pumice Stone

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ABSTRACT: *In this study, Remazol Yellow (RR) removal was investigated by Saccharomyces cerevisiae immobilized on pumice stone. For immobilization process new technic was used and immobilization matrix HCl pretreated pumice stone was added to growth medium of microorganism. pH, initial biosorbent (C_o) and dye concentration (C_b) effect on biosorption were optimized through the Response Surface Methodology (RSM) and second order quadratic model was used to describe the effects of parameters successfully. At optimum conditions pH 3, C_b 2.5 g/L, C_0 400 ppm maximum dye removal was 99% and 140 mg/g capasity was catched. When pumice stone, HCl preated pumice stone and Saccaharomyces cerevisiae were used in biosorption experiments directly 44%, 69%, 75% dye removal was obtained respectively. 0.5 M NaOH (pH 13.69) and water (pH 8) were chosen as a desorption agent for the immobilised biosorbent. Desorption efficiency was found 21% with 0.5 M NaOH and 1.5% with water (pH 8). The characterization studies were performed by using Scanning Electron Microscope (SEM) and Fourier Transformer InfraRed (FT-IR) spectroscopy. The results indicate that immobilised biosorbent is a promising alternative for the biosorption of Remazol Yellow (RR) from aqueous solutions.*

KEYWORDS: *Immobilization; S. cerevisiae; Remazol Yellow (RR); Pumice Stone.*

INTRODUCTION

Dyes, even at very low concentrations, reduce wastewater transparency and oxygen solubility moreover, these chemicals are toxic, carcinogenic or mutagenic for various organisms [1]. Synthetic dyes are great environmental concern due to their widespread usage and their low removal rate during aerobic waste treatment. About 10.000 different dyes are prepared globally and approximately 8×10^5 tons of synthetic dyes are consumed in textile industries in the whole world and it is necessary to reduce its content in industrial effluents before their discharge into environment [2,3].

Synthetic dyes can be divided into acidic, reactive,

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direct, basic and other groups. Remazol dyes from reactive dyes group are very popular and relatively low sustantivity made them especially useful for printing [4]. Remazol RR dyes can be used as true reactive dyes on cotton, silk, and wool which is widely used in textile industries because of easy process and low temperature needing but at the end of the dying process 10-15% of the initial dye load is supplied by from dyebaths to wastewater. Without appropriate treatment, the release of such wastewater into the environment is a serious problem for aquatic environment and human health [5].

Various chemical-physical methods like flocculation, coagulation, membrane filtration and surface adsorption are used for decolorizing textile wastewaters[6]. Although these methods are efficient for treatment of waters contaminated with pollutants, they are very costly and commercially unattractive [7,8]. Recently, research attention has been focused on biological methods. In a simple way, biosorption may be defined as the removal of substances from solution by biological material which can be organic or inorganic, in gaseous, soluble or insoluble form. The key advantage of biosorbent is cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind dye ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups which accounts for the majority of dye adsorption by ion-exchange mechanism. Physical characteristic of biosorbent (particle size, density, mechanical stres, etc.) cause some problems and to overcome these problems the use of immobilization technics is the best way. Immobilization can be defined as ‘‘the physical confinement or localization of cells to a certain defined region of space with preservation of some desired activity’’ [9]. Immobilization has some advantages like better stability, increased activity and selectivity, higher resistance, improved separation and purification, reuse of biosorbent, and conse-quently more efficient process [10]. Ideal immobilization support properties are described to include insolublity, high mechanical stability, high diffusivity, simple immobilization procedure, minimal attachment of other organisms and preferably a low cost price [11].

Pumice stone, which is commonly found in Turkey and thus is easily and cheaply accessible is a good alternative material for immobilization [12]. Pumice stone is a type of light, highly porous (pore volumes up to 85%) and lowcost volcanic stone with high silica content. On account of its highly porous structure, it provides a large number of possible attachment sites for immobilization [13]. Previously several researchers had proved some studies; *Saccharomyces cerevisae* immobilized pumice stone for removal of Fe2+ from aqueous solution [14], Penicillium digitatum immobilized on pumice stone for removal of Cu(II), Zn(II) and Pb(II) ions [15], chitoson immobilised pumice stone for sorption of As(V) from waters and results demonstrate the advantage of immobilizing chitosan onto pumice stone with 90% sorption capasity [12].

Experimental design technique is a very useful tool, as it provides statistical models which help in understanding the interactions among biosorption parameters (like pH, contact time, initial concentration and adsorbent dosage) that have been optimized. RSM helps to determine and optimize effective parameters condition by minimizing the number of experiment. The method was introduced by Box and *Wilson* in 1951 [16]. *Danda et al.* used rice husk ASH for removal of nickel and used the statistical design to optimize process conditions [17]. *Radaei at al.* used RSM for removal of Reactive Blue 19 from aqueous solution [18]. *Zulhelmi at al.* used RSM successfully to investigate the parameters (dosage of biosorbent, pH of the metal solution and contact time) that influenced the removal of lead(II) and nickel(II) ions from the solution [19]. Deepa at al. studied the biosorption of Pb (II) by using the leaves Araucaria cookii from aqueous solution and pH, contact time, metal ion concentration, adsorbent dose, and particle size variation optimised by RSM method [20].

According to literature review, few study on immobilization of *S. Cerevisiae* to pumice stone by adding pumice stone to growing medium of microorganism for Remozol dyes removal has been performed, also there are very few research about *S. Cerevisiae* immobilization with different immobilization matrix for waste water treatment. The aim of this work was to evaluate the Remazol Yellow (RR) removal by thermally dried *S. cereveisiae*, pumice stone, surface modified pumice stone and immobilised biosorbent which was obtained by a new method that adding the surface modified pumice stone to the growing medium of *S. Cerevisiae*. Optimum biosorption parameters pH, C_o and C_b which were obtained through RSM. Desorption studies with 0.5 M NaOH (pH 13.69) and water (pH 8) were carried out to see reusability of the immobilized biosorbent. The characterization studies

were performed by using FT-IR and SEM before and after the biosorption.

EXPERIMENTAL SECTION

Chemical Reagents

The chemicals for the agar-malt extract such as the malt extract, yeast extract, peptone, glucose, agar were purchased from Sigma Aldrich, microbiological part. Growth medium chemicals were; $(NH_4)_2SO_4$ (Sigma Aldrich, ≥99.0 %), KH_2PO_4 (Aldrich, 99.99%), $CaCl_2$ (Sigma, Anhydous, ≥ 96.0%), $MgSO_4.7H_2O$ (Sigma, 99.5 %), $(NH4)_2SO_4$(Sigma Aldrich, ≥99.0 %), H_2SO_4(Sigma, ≥ 98.0 %), HCl (Sigma, ≥ 98.0 %).

Remazol Yellow (RR) was supplied by Dystar which is a leading dyestuff & chemical manufacturer and solution provider. An accurately weighed quantity (1 g) of Remazol Yellow (RR) was dissolved in double distilled water to prepare stock solution of 1000 mg/L.

Immobilization Process

For immobilization process immobilization support matrix pumice stone was sieved approximately 500 µm mesh. Before adding to the growth medium of *S. cerevisiae*, pumice stone was pretreated with HCl. For the pretreatment of pumice stone, 5 g of pumice stone was added to 100 mL 1 N HCl and waited 24 hours at room temperature. It was filtered and washed with distilled water many times until pH became constant (pH=4.5) and dried at 50 °C.

S. cerevisiae was taken from the Turkish Public Health Center. Every month fresh agar slants were perapared, microorganism transfered on this agar and stored at 4 °C. Agar-malt extract contained (g/L): malt extract 3.0; yeast extract 3.0; peptone 5.0; glucose 10; agar 20; pH 4.5. At starting point of each experiment the yeast was subcultured on agar-malt extract slants. Culture was grown in an incubator at 30 °C for 24 hour. For the seed culture, the inoculum from the fresh slant culture were transferred aseptically to previously sterilized 500 mL growth medium. The growth medium contained (g/L); glucose 50.0, yeast extract 5.0, $(NH_4)_2SO_4$, KH_2PO_4 12.0, $CaCl_2$ 0.5, $MgSO_4.7H_2O$ 1.0, $(NH_4)_2SO_4$ 5.0, yeast extract 5.0, $CaCl_2$ 0.5. The pH of the medium was adjusted to 4.5 with 0.5 M H_2SO_4. For immobilization process, HCl pretreated pumice stone was added to this growth medium of 30 °C and agitated using a magnetic stirrer at 440 rpm. After approximately 16 h corresponding to the mid exponential growth phase, solid phase from liquid was separated using a centrifuge at 7000 rpm and solid phase was washed several times with deionized water. Non-living biomass was obtained by autoclaving cultures at 121 °C for 30 min and dried at room temperature. The dried biomass was ground and sieved through a 200 mm mesh and stored in a desiccator [21].

Biosorption Experiments

Biosorption experiments were conducted using 250 mL erlenmeyer flasks to which 100 mL of dye containing waste water and biosorbent were added. These biosorption flasks were agitated in a temperature–controlled orbital shaker at a constant speed of 140 rpm. pH was adjusted by addition of 0.1 M H_2SO_4. During biosorption experiments samples were withdrawn at appropriate time intervals and these samples were centrifuged at 6000 rpm for 10 min and the supernatant fraction was analyzed for the residual dye concentration.

For analytical measurements the concentration of the dye Remazol Yellow (RR) were determined using a UV-Vis spectrophotometer (HITACHI U 2000, spectrophotometer) at a characteristic wavelength corresponding to the maximum absorbance of the dye (416 nm) until at which no further decrease in the dye concentration was measured.

The amount of dye removal was calculated from the following equation:

$$\%\,\mathrm{Removal} = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

Where C_o was the liquid phase concentrations of the dye at initial (mg/g), C_t was the the liquid phase concentrations of the dye at time *t*.

Desorption Study

The biosoption study of biosorbent is not only on the dye removal percentage and binding the greatest possible amount of biosorbent, it also on how the biosorbent can be regenerated and used again. To investigate the reusability of biosorbent, desorption experiments were conducted, Remazol Yellow (RR) is an acidic dye and for desorption studies basic medium was prepared at two different pH values 0.5M NaOH (pH 13.69) and water (pH 8 adjusted).

To investigate the desorption efficiency, Remazol Yellow (RR) loaded immobilised biosorbent was filtered from equilibrium biosorption solution and dried. Then it was taken in 50 mL of desorption agent and agitated for the equilibrium time of Remazol Yellow (RR). At the end, the liquid phase was filtered and analyzed then desorption efficiency was calculated from the following equation;

$$\text{Desorption efficiency} = \frac{C_{des}}{C_{ads}} \times 100 \quad (2)$$

where C_{des} (mol/L) and C_{ads} (mol/L) are respectively the concentrations of desorbed and adsorbed dye ions in equilibrium by the biosorbent [22].

Characterization

FT-IR spectrum is the feature of a particular compound that gives the information about its functional groups, molecular geometry and inter/intra molecular interactions. FT-IR spectrums of the biosorbent was collected using Shimadzu IRTracer-100 instrument. The FT-IR analysis of the immobilized biosorbent before and after the biosorption was performed between 600-4000 cm^{-1} wavelength.

The morphology and features of biosorbent surface can be investigated with the help of a SEM, which can be utilized to find out the porous structure of biosorbent and also give information about particle shape or size [23]. The difference in the surface morphology of the biosorbent before and after the biosorption was observed by using Carl Zeiss/Supra 40VP instrument and recorded with two different magnifications, i.e. 2000 and 5000 times.

Experimental Design Method

Application of RSM is sequential in nature, and optimization is done through three successive major steps such as (1) designing of experiments, (2) analyzing the responses with prediction of model and (3) finding out optimum condition. RSM tells us the relationship between diferent input factors and the response. Mathematically, it can be shown as follows:

$$Y' = f\left(X_1', X_2', \ldots, X_n'\right) \quad (3)$$

Where Y' is the response and, (X'_1, X'_2, ……. X'_n n are the independent variables called the numeric factors. Central Composite Design (CCD), the most familiar class of second-order design, was used in the present study.

pH (X_1), C_b(X_2) and C_o(X_3) were considered as independent variables and % dye removal was dependent parameter for the process.

Thus, for n number of variables, total number of tests required is:

$$N = 2^n + 2n + n_c \quad (4)$$

For the three input variables used in this study, the total number of tests required is:

N = 23 +(2 × 3)+6 = 20.

To avoid aliased terms present in the higher-order models, a second-degree polynomial equation is selected to analyze the responses as a function of numeric factors. The equation is as follows:

$$Y' = \beta_0 + \sum \beta_{ii} X_i'^2 + \sum \beta_{ii} X_i' X_j' \quad (5)$$

Where β_0 = the constant coefficient, β_i = the slope or linear effect of the input factor X′i , β_{ii} = the quadratic effect of input factor X'_i , β_{ij}=the linear-by-linear interaction effect between the input factors X′ and X'_j. [24].

The analysis of variance (ANOVA) was used to evaluate the statistical significance of the constructed models. Results were evaluated in Design Expert 9.0 (Stat-Ease, Inc. USA) trial version statistically with ANOVA analysis.

Independent variables, pH (X_1) was varied between 2 and 4 (ΔX_1=2), C_b (X_2) 1.5-3.5 mg/L (ΔX_2=1.5 mg/L) and C_o(X_3) 200-600 ppm (ΔX=200 ppm). The experiments consist of six axial (A), eight factorial (F) and six center points. The center point was repeated four times. Computation was carried out using multiple regression analysis using the least squares method. Value of readability alpha (α) is important to calculate as it could determine the location of axial points in experimental domain. Depending on alpha value, design is spherical, orthogonal, rotatable, or face centered.

Choice of α depends on the number of variables k and α is calculated from $2^{k/4}$. If alpha is greater than unity, it means it becomes spherical and α is calculated from $k^{1/2}$ and α found as 1.73. [25] For every independent variable X_i, coded value U_i was calculated with the use of following equation reference;

$$U_i = \frac{X_i - X_{i0}}{\Delta X_i} \quad (6)$$

Table 1: Independent variables in experimental plan.

X_i	Factor levels				
	- 1.73	- 1	0	+1	+1.73
pH, X_1	1.27	2	3	4	4.73
C_b, X_2 (mg/L)	0.77	1.5	2.5	3.5	4.23
C_0, X_3 (ppm)	53.54	200	400	600	746.41

Where X_{io} was the mean value of independent variables, X_i was the real values of independent variables, ΔX_i was the step size.

Values of independent variables in experimental plan is given in Table 1.

RESULTS AND DISCUSSION

In this study, pH, initial biosorbent (C_o) and initial dye concentration (C_b) effect on biosorption were optimized through RSM and RSM model fitted well to the emprical quadratic model.

In Table 2 coded and real values of independent variables and also predicted and observed % dye removal values are given. Percentage error was calculated from calculated and experimental data.

The results of ANOVA are shown in Table 3. The significance of each coefficient was determined using the F-test and p-value. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller [26].

The Model F-value of 45.54 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case pH, C_b, C_o, pH^2, C_b^2, C_o^2 are significant model terms for this biosorption process and by the removal of the insignificant terms, the fitted quadratic model for percent biosorption in coded variables is given in this equation;

$$\%\text{Biosorption} = -150.2 + 81.77 \times \text{pH} + 97.62 \times C_o + 0.18 \times C_b - 18.13 \times \text{pH}^2 - 15.63 \times C_0^2 - 0.004 \times C_b^2 \quad (7)$$

The model gives the real relationship between independent variables and the response. The correlation coefficient (R^2) provides a measure of the models variability in the observed response values. The closer the R^2 value to 1, the stronger the model is and it predicts the response better. Regression coefficient for the quadratic model was found 0.98 and it showed that model can explain us all the changes in the response clearly. "Adj R-Squared" must be greater than 0.7 and it was 0.9547 also the "Pred R-Squared" of 0.8131 was in reasonable agreement with the "Adj R-Squared" of 0.9547; the difference is less than 0.2 [27]. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The resulted ratio of 19.91 indicated an adequate signal.

Relationship between actual and predicted values were showed on Fig.1. The data points for the biosorption process are distributed near the straight line showed that the quadratic model is predictions are statistically a satisfactory match with the observed values.

Therefore, this model can be used to navigate the design space. In fact, Fig. 1 depict the relationship between the values of R^2 and Adj R-Squared which shows a very good agreement between the predicted and actual data.

Graphical representation of regression equation is represented by three-dimensional (3D) response surface. It shows the relationship between responses and experimental levels of each variable as well as the type of interactions between the two tested variables.

Three-dimensional plots for percent biosorption as the function of pH, C_b and C_0 are given in Fig. 2(a)-(c). These plots served as a very good tool for analyzing the interaction effects of the dependent variables.

The percent biosorption as a function of pH and C_b is shown in Fig. 2(a). Maximum % removal was obtained at 2.5 g/L C_b and pH 3. Percentage of dye removal increases with decreasing pH and increasing C_b. At lower pH, the surface of the biosorbent will react as positively charged, and hence, effectively attracts the negatively charged anionic dye, leading to an increased sorption of acid dyes. At higher pH, the surface of the biosorbent becomes negatively charged, which reduces the interaction of the anionic dye. In previous literature, similar observations have been reported about pH effect on biosorption process

Table 2: Coded and real values of independent variables and also calculated and experimental % removal values.

Run	pH	C_b	C_0	% biosorption (observed)	% biosorption (predicted)	Error
		g/L	ppm			
1	4	3.5	600	12	2	5
2	3	2.5	400	98	87	0
3	2	3.5	600	41	56	0
4	3	4.23	400	89	74	0
5	4	1.5	200	32	18	1
6	4.73	2.5	400	14	13	0
7	4	1.5	600	5	6	0
8	3	2.5	400	99	87	0
9	2	3.5	200	99	97	0
10	3	2.5	400	98	87	0
11	3	0.77	400	22	7	2
12	2	1.5	600	21	17	0
13	3	2.5	400	97	87	0
14	4	3.5	200	71	57	0
15	1.27	2.5	400	82	80	0
16	3	2.5	53.54	99	87	0
17	2	1.5	200	80	72	0
18	3	2.5	746.41	9	8	0
19	3	2.5	400	99	87	0
20	3	2.5	400	98	87	0

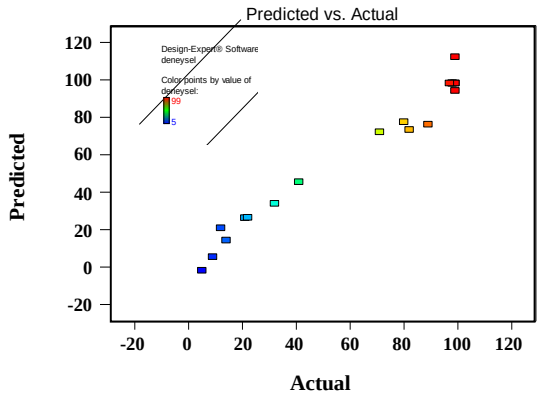

Fig. 1: Relationship between and actual and predicted values.

that for acidic dyes percentage dye removal increases with decreasing pH [28-33].

Fig. 2(b) shows the response as a function of pH and initial dye concentration. Maximum % removal was obtained at pH 3 and 400 ppm dye concentration. % dye removal decreases with increasing C_o and pH. The dye removal efficiency is highly dependent on the initial dye concentration. The effect of initial dye concentration relies on the immediate relation between the dye concentration and the available binding sites on the adsorbent surface. The removal efficiency will decrease with an increase in the initial dye concentration due to the saturation of adsorption sites on the adsorbent surface [34]. There will be

Table 3: Analysis of variance table ANOVA for RSM parameters.

	Sum of		Mean	F	p-value		
Source	Squares	df	Square	Value	Prob > F		
Model	26918.99	9	2991.00	45.54	< 0.0001		significant
pH	4072.54	1	4072.54	62.01	< 0.0001		
C_b	2887.15	1	2887.15	43.96	< 0.0001		
C_0	9199.87	1	9199.87	140.08	< 0.0001		
pH* C_b	6.13	1	6.13	0.093	0.7663		
pH* C_0	120.13	1	120.13	1.83	0.2060		
C_b* C_0	120.12	1	120.12	1.83	0.2060		
pH^2	5175.25	1	5175.25	78.80	< 0.0001		
C_b^2	3846.19	1	3846.19	58.56	< 0.0001		
C_0^2	4096.25	1	4096.25	62.37	< 0.0001		
Residual	656.76	10	65.68				
Lack of Fit	653.92	5	130.78	230.80	< 0.0001		
Pure Error	2.83	5	0.57				
Cor Total	27575.75	19					

will be unoccupied binding sites on the adsorbent surface at a low dye concentration, and when the initial dye concentration increases, there will be insufficient sites for the adsorption of dye molecules, thus decreasing the dye removal efficiency

Similar results by some researchers have been obtained in this regard that percentage dye removal increases with decreasing C_b [34-37].

In Fig. 2(c) maximum % dye removal was found at C_b 2.5 g/L and C_o 400 ppm. % dye removal increases with decreasing C_o and increasing C_b. Generally the dye removal increases with increasing adsorbent dosage, where the amount of sorption sites at the surface of adsorbent will increase by increasing the dose of adsorbent, and as a result increase the percentage of dye removal from the solution. In literature similar trend was observed by researcher [38-41].

In experimental part, at optimum working conditions pH 3, C_o 400 ppm and C_b 2.5 g/mL which were obtained from RSM, pumice stone, surface modified pumice stone and thermally dried *S. cerevisiae* were used in biosorption experiments directly and 44%, 69%, 75% dye removal was obtained respectively. As shown from Fig. 3 obtained removal percentage of HCl pretreated pumice stone was better than unpretreated form. It is known that the main component of the pumice stone is SiO_2 this component can discompose to Si^{+2}, leading to the formation of Si^{+2} species at the surface of the biosorbent. In aqueous solution acid dyes is first dissolved and then discomposed to the sulphonate groups of acid dye and anionic dye groups as following:

$$DSO_3Na \rightarrow DSO_3^- + Na^+ \quad (8)$$

Sulphonate groups of acid dye with $Si^{+}2$ due to electro-static force created:

$$Si^{+2} + DSO_3^- \rightarrow Si(DSO_3)_2 \quad (9)$$

When pumice stone is pretreated with HCl, H^+ ions is added to the surface of pumice stone and this increases the cationic capacity;

$$H^+ + Si^{+2} \leftrightarrow HSi^{+3} \quad (10)$$

Also pumice stone shows smaller pore structure after treatment by HCl. The initial specific surface of pumice stone is increased after acidic treatment [42].

Remazol Yellow (RR) is acidic dye and cationic effect on pumice stone with HCl increases the percent removal

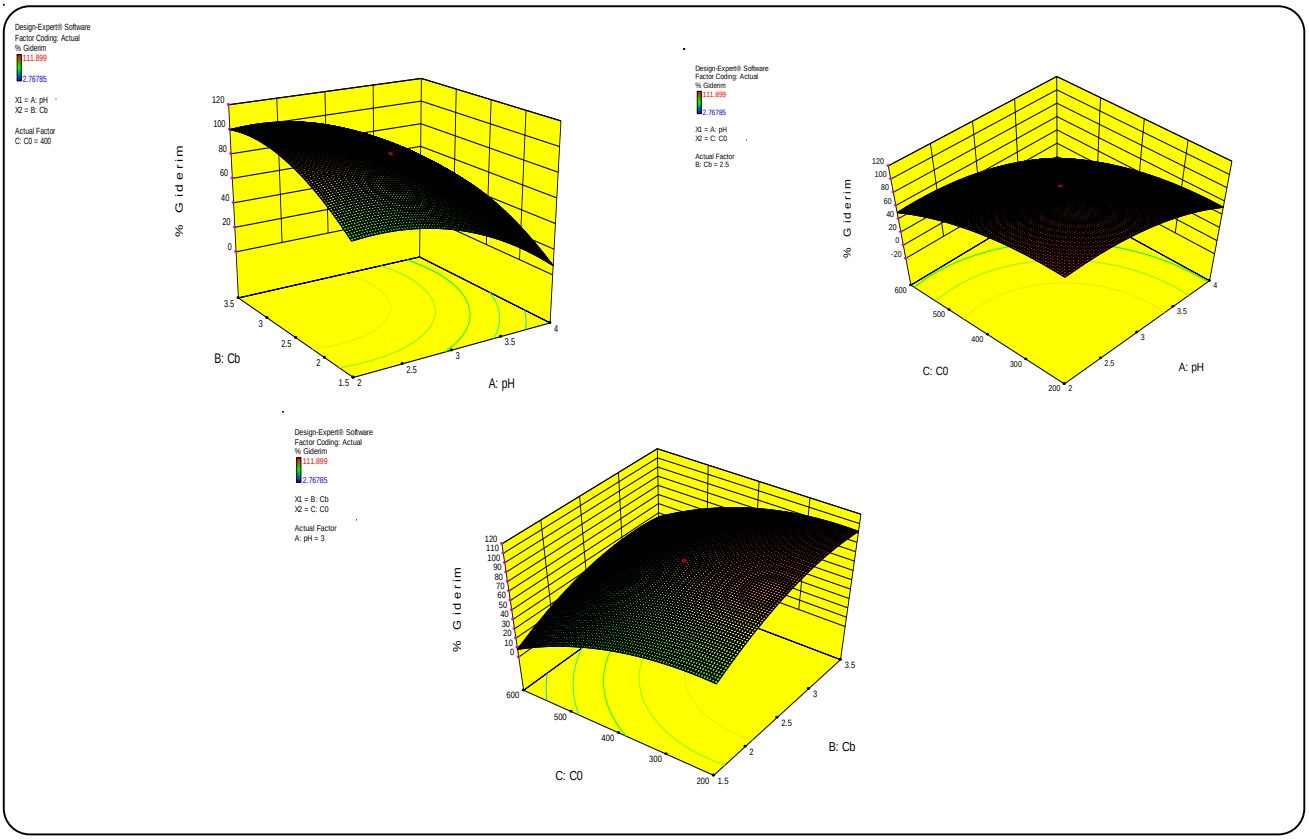

Fig. 2: a) Influence of pH and C_b on percent removal b) Influence of pH and C_o on percent removal c) Influence of C_b and C_o on percent removal (pH 1.27-4.7, Co 53.54-746.41 ppm, Cb 0.77- 4.23g/L)

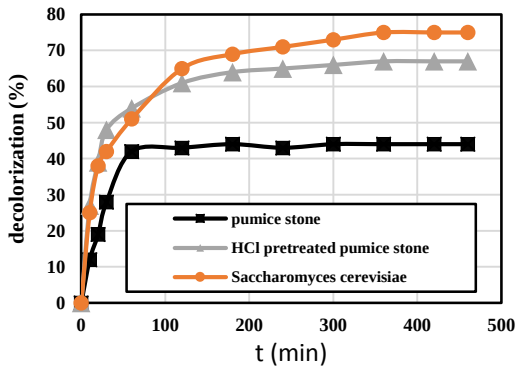

Fig. 3: % removal at optimum operating conditions with HCl pretreated pumice stone (pH=3; C_0= 400 ppm; C_b= 2.5 g/L; T = 25 °C).

of dye and effects the biosorption positively. Similar observation was reported by [42].

75% dye removal was found with *Saccaharomyces cerevisiae*.

The percentage of dye removal with immobilised biosorbent is shown in Fig. 4. 99% dye removal was catched and according to this calculated result from Anova was confirmed with experimental result and 99% dye removal was found. Adding the immobilization matrix pumice stone to the growth medium of *Saccaharomyces cerevisiae* let cells to diffuse porous structure of the pumice stone when they are growing. Immobilization step improve the dye removal ability of *Saccaharomyces cerevisiae* and pumice stone.

In literature when thermally dried *S. cerevisiae* was used as a biosorbent 95%, 96.16%, 61.82%, 96%, 97% dye removal percentage were achieved for Remazol Turkuaz, Navy Blue, Remazol Blue, Red 33:1 and Orange 1. When pumice stone was used as a biosorbent percentage dye removal was 38% for Neutral Red, 75% for AR14, 70% for AR18 [47-49]. When *S. cerevisiae* immobilized on sugarcane 31.34% percentage of dye removal for acid black 48, when *S. cerevisiae* immobilized on sodium alginate 90% percentage of dye removal for mono-azo dyes and nearly 50-60% for di-azo dyes [50,51].

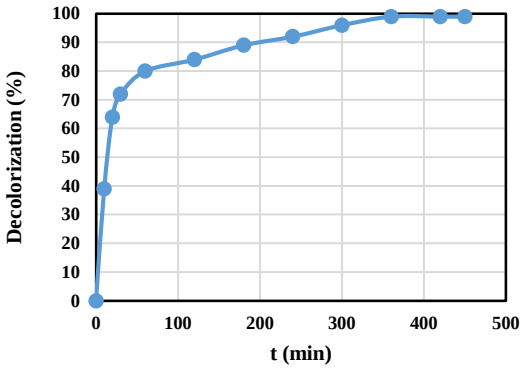

Fig. 4: % removal at optimum operating conditions with immobilised biosorbent (pH=3; C_0= 400 ppm; C_b= 2,5 g/L; T = 25 °C).

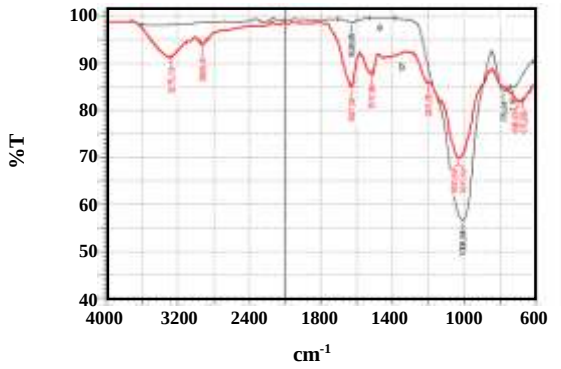

Fig. 5: FT-IR spectra of immobilised biosorbent before (a) and after the biosorption (b).

In this study, with new immobilised biosorbent high dye removal percentage was catched. It showed that immobilization step changed and improved the surface of *S. cerevisiae* and pumice stone positively.

Characterization

The FTIR analysis of the immobilized biosorbent before and after the biosorption was performed between 600-4000 cm^{-1} wavelength and the functional group interaction of biosorbent-dye was examined in Fig. 5.

In the FT-IR spectrum of unloaded immobilised biosorbent before biosorption (Fig. 5a), the band at 1629.85 cm^{-1} corresponded to C=O Stretching Vibrations, 1006.84 cm^{-1} corresponded to C-OH Stretching Vibrations, 779.24 cm^{-1} shows aromatic compounds.

At Fig. 5(b) the different new bands appeared with dye loaded immobilised biosorbent; the band at 3275 cm^{-1} corresponded to N-H Stretching Vibrations, 2926 cm^{-1} corresponded to C-H Stretching Vibrations, 1517 cm^{-1} corresponded to N=O Stretching Vibrations and 1201 cm^{-1} corresponded to C-O Stretching Vibrations.

Significant band shifted on the surface of the immobilised biosorbent after biosorption, from 1629 to 1627 cm^{-1} . This decrease in the wave number of the peak characteristic for C=O group from carboxylic acid revealed that interacts with carbonyl functional group are present between immobilised biosorbent and dye ions. Other shifted bands were from 1006 cm^{-1} to 1031 cm^{-1} and from 779.24 to 698.23 cm^{-1}.

These shifts in the wavelength showed that there was dye binding process taking place at the surface of the immobilised biosorbent.

When the obtained FT-IR peaks are examined, the formation of some new peaks or shift of existing peaks indicates that the immobilized biosorbent retains Remazol Yellow (RR) dye molecules and consequently some structural changes occur.

SEM images of immobilised biosorbent shows maximum % removal were taken at different magnifications, before and after biosorption. As presented in Figs. 6(a) and (b) immobilised biosorbents has well-defined and regular porous structures like honeycomb which has suitable empty places for the dye ions. After biosorption as seen in Figs. 6(c) and (d), the pores were filled completely with adsorbed molecules and surface morphology of the immobilised biosorbent changed with interaction between dye molecules and cell wall.

Desorption Study

Desorption efficiency of immobilised biosorbent was found 21% with 0.5 M NaOH and 1.5% with water (pH 8) at the first biosorption–desorption cycle. A low desorption of a dye may suggest that the physical biosorption was not prevalent in the biosorption process. Similar low desorption lower amount of desorption trend was observed by [52-54].

CONCLUCIONS

In this study, HCl pretreated pumice stone was added the growing medium of *Saccaharomyces cerevisiae*

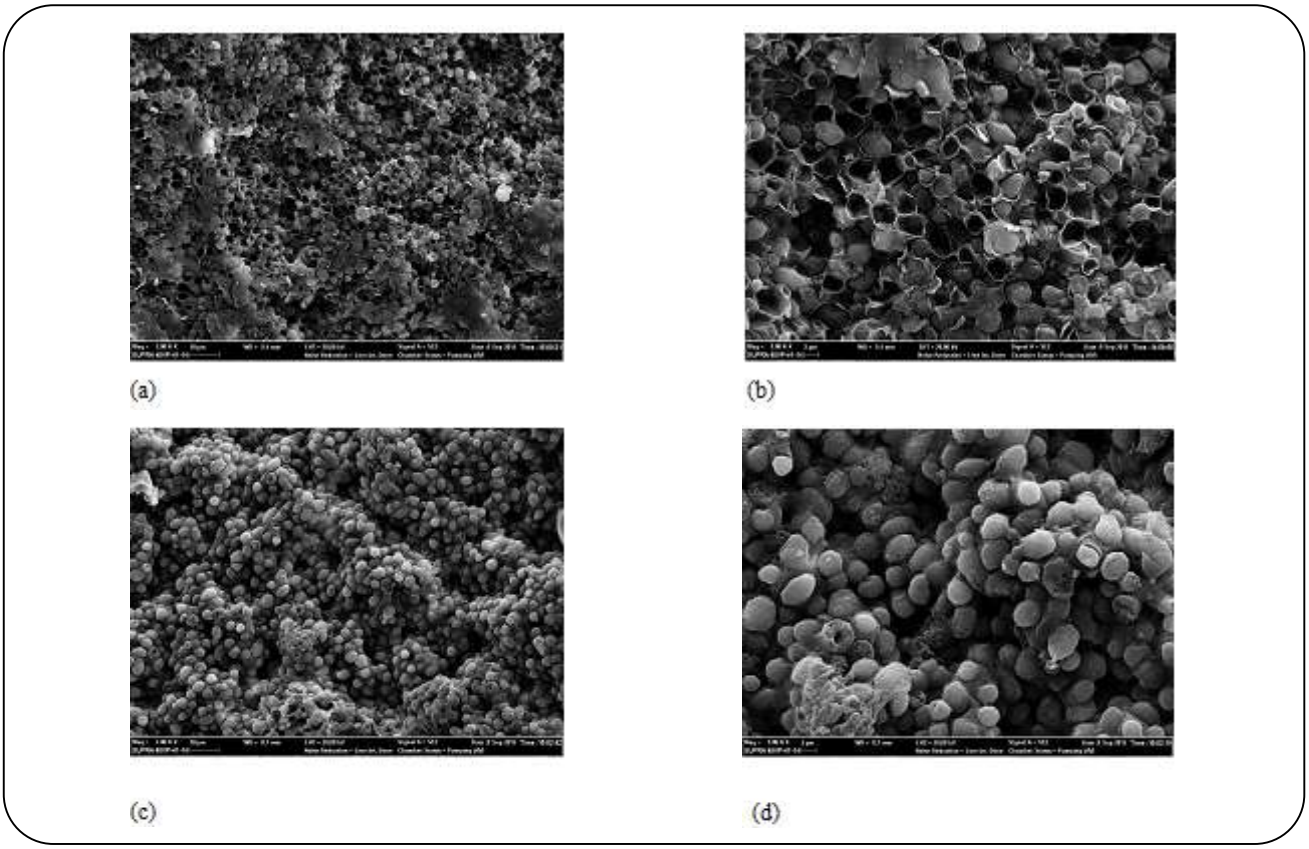

Fig.6: SEM images of immobilised biosorbent before biosorption (a) x2000, (b) x5000 and after biosorption (c) x2000 (d) x5000.

and at optimised process parameters pH 3, C_b 2.5 g/L ve C_0 400 ppm 99% Remazol Yellow (RR) removal was obtained. Quadratic models derived from RSM were successfully predicting all the responses. ANOVA showed that change of pH, biosorbent dosage and dye concentration effect the dye removal significantly. When the pumice stone, HCl preated pumice stone and *Saccaharomyces cerevisiae* were used directly in biosorption process lower dye removal values were obtained 44%, 69%, 75%. Results showed that HCl pretreatment of the pumice stone is good alternative method for improving biosorption capasity of pumice stone and immobilization step improve the biosorption capacity of pumice stone and *Saccaharomyces cerevisiae.*

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